

Project No. _____

Book No. _____

TITLE 100gram Crack Tne

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Growth: 764 D1 00 IR -100g

Slurry cells in 200 mL of crack butter - lg: 2 mL
Final Volume - 300 mL

Crack butter -
25 mM Tris pH 7.4
1 mM EDTA
1 mM PMSF
8% glycerol
5 mM BME

Filter cells slurry through 4 layers
of cheese cloth

Pass through gauze (mesh) 2x @ 100

Set up 90°C heat bath before cracking cells. Use -
Floor Shaker -

Innoculate @ 85°C for 12 minutes - with light shaking -
cool immediately on ice water bath ~15 min -

Spin @ 18,000 xg in GSA rotor - collect supernatant -
- 40 min - bright yellow color -

PEI precipitation - 4% PEI + 50 mM KCl final concentration
add slowly over 20 minutes

let stir an additional 45 min - spin down @ 18,000 xg
in GSA rotor - 40 minutes -

N.S. (NH₄)₂SO₄ precipitation -

Add (NH₄)₂SO₄ solid to a final of 60% saturation -
Add slowly over 30 minutes - let stir o/n @ 4°C

100 mL : $\frac{17}{250}$

T Pag N

Witnessed & Understood by me,

Date

Invent d by

Dat

May Lopez

4/5/95

E. Flynn

Rec rd d by

03/29/95